Demodex folliculorum: Requirements for Understanding Its Role in Human Skin Disease

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The data and text commentary herein were drawn posthumously from laboratory notebooks and writings found in Dr. Kligman's study and bedroom. Comments in brackets were added by Barbara A. Gilchrest (Department of Dermatology, Boston University School of Medicine) and James J. Leyden (Department of Dermatology, University of Pennsylvania School of Medicine).

Demodex folliculorum was first described independently by two investigators nearly 170 years ago. Human beings are the sole host for this mite (Supplementary Figure 1 online), which resides exclusively in sebaceous follicles and has been repeatedly but inconclusively implicated in rosacea by more than 1,000 peer-reviewed papers, approximately half of them published since 1990. Sometimes visible within follicles in routine cross-sectional biopsy sections, Demodex is more reliably detected in "skin surface biopsies," a technique first employed by Marks and Dawber (1971) and modified by Mills and Kligman (1983). The purpose of this investigation is to further refine the technique to make it more accurate, reproducible, and quantitative—characteristics necessary for implicating the mites in skin disease. [*One of Kligman's characteristics was to periodically start over and assume everything previously said or published was probably wrong, even if it was he who had said or published it. At times in the past he had dismissed the possibility that Demodex has a role in skin disease, but at the end of the trail he reversed his position and determined to seek data in support of the possibility. Thus, with his longtime collaborator, Mike Christensen, Kligman embarked on a bottom-up re-exploration of whether Demodex plays a role in skin disease. True to his lifelong approach, Kligman decided to begin by improving, if possible, the technique for quantifying Demodex.This methodology is described online.]*

METHODS

Subjects

Surface biopsies of facial skin were obtained from 20 healthy women aged 27–50 years (median 44 years). The study was approved by an institutional review board, and informed written consent was obtained. No medications or cosmetics were applied to the cheeks during a run-in period of 2 weeks.

Surface biopsy technique

Subjects briefly washed their faces with a bland soap and water and then put on a pair of safety goggles. A drop (about 0.05 ml) of cyanoacrylate glue (Krazy Glue) was applied to one end of a plastic slide (1 × 3–inch plastic slides; Rinzl) and spread out to a uniform thickness, using the nozzle of the Krazy Glue bottle. The slide was then pressed against the medial cheek, causing the glue to spread to a thin film the width of the slide (1 inch) and approximately half its length (1.5 inch). The slide was left in place for 5 minutes while the cyanoacrylate hardened as it polymerized. Before removal, an outline of the slide was drawn with a Sharpie permanent marker, leaving a demarcated area on which a second cyanoacrylate-coated slide was applied. Two sequential surface biopsy samples were thus obtained, taken from precisely the same site, a procedure that caused minimal discomfort and erythema.

Slides were placed face up on the stage of a stereomicroscope for quantitative assessment of horny casts and determination of mite density per square centimeter. A second biopsy is shown in Supplementary Figure 1. To quantify mite densities, with the slide in place the microscope stage was moved under the objective lens to generate five distinct representative fields. The casts in each were counted separately, and a mean of the five values was used to calculate casts per square centimeter.

To liberate the mites from the casts, a drop of olive oil was placed on the slide, sufficient to cover the entire sample. Casts (a total of 20, randomly selected) were then disrupted individually with a fine needle. As each cast was disrupted, the number of mites released was immediately counted while the mites swam freely in the olive oil medium (Supplementary Figure 2). Mites remained mobile in the olive oil for nearly 3 hours, in contrast to less than 2 hours in mineral oil, before becoming motionless and more difficult to distinguish from cellular debris. Even when large numbers of casts were found on a biopsy, not all casts contained mites, so we determined two factors after counting: the percentage of casts infested and the mean number of mites per infested cast. Using these two factors and the number of casts per square centimeter, we calculated the number of mites per square centimeter

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as follows: casts/cm^2 × the fraction of casts infested × mean number of mites/infested cast = number of mites/cm^2. The combined yields from the first and the second biopsies were defined as the total burden of mites per square centimeter of skin. In two subjects, taking a third sequential biopsy from the same site proved to be painful and produced an erythematous reaction that persisted to the next day. Mites could still be recovered, although far fewer in number than in the second biopsy, suggesting that the two-sequential-biopsy technique only modestly underrepresent the true mite burden.

RESULTS

The density of mites on the face ranged from 0 to 384 mites/cm^2, with a mean of 214 mites/cm^2 (Supplementary Table 1). The values determined for each of the two sequential surface biopsies taken from each subject were comparable, averaging 104 and 110, respectively, and thus taking two sequential biopsies doubled the yield of mites isolated from each subject's facial skin. This doubling of the yield of mites occurred despite a significant reduction ($P < 0.0005$) of 33% in the number of infested casts per square centimeter in the second versus the first biopsy—23.6 versus 35.3 per square centimeter of skin surface—because of compensatory increases in the percentage of casts infested and in the mean number of mites per infested cast, both of which were significantly higher in the second biopsy than in the first one.

There were no significant differences between the left and right cheeks in the five subjects studied (data not shown). This apparent symmetry of mite distribution suggests that a split-face study design would be appropriate for testing mite eradication via topical ascaricidal formulations on the face. [*Kligman, thinking ahead to the clinical implications of all experimental findings!*]

In the two subjects with no mites detected after two surface biopsies, 3-mm punch biopsies were obtained from the opposite cheek and serially sectioned parallel to the skin surface and then stained with periodic acid–Schiff to reveal mites. [*Kligman's persistence at work!*] Clusters of mites were present in the deep infundibular portion of the follicular canals in both subjects. In contrast, in two subjects with high mite counts (>270/cm^2) on surface biopsy, horizontally sectioned punch biopsies revealed mites throughout the canal. This suggests that follicles in all adults are infested, but that the distribution of mites within the follicular canal varies; this variation may lead to occasional false-negative results when using this surface biopsy technique due to sequestering of mites deep in the canal.

Although not quantified, the density of horny casts (compaction and number of desquamated follicular cells) was roughly proportional to the mite count per square centimeter, suggesting that the abundant proliferation of mites promoted cast formation. In support of this interpretation, in histologic sections sebaceous follicles containing small numbers of mites did not show a hyperkeratotic response whereas those containing numerous mites often did. [*This sentence may be read as Kligman's invitation to future investigators to pursue these "twentieth-century" histologic observations, using molecular techniques to probe the pathophysiology of rosacea and other mite-associated skin disorders.*]

DISCUSSION

The literature contains many observations consistent with a pathogenic role for Demodex in rosacea (Schmidt and Gans, 2004; Rufle and Buchner, 1984; Georgala *et al.*, 2001; Lacey *et al.*, 2007; Grosshans *et al.*, 1980; Ayres and Ayres, 1961). There have been numerous reports of high mite counts in affected skin, and ascaricidal drugs often ameliorate the disease. These include lindane, permethrin, ivermectin, and sulfur. Further, in cats and dogs the skin disease "mange" is well documented to be caused by mites and can be treated successfully with ascaricidal agents (Schwartzman, 1976; Mather 1980).

[Of note, an important recent advance in understanding the pathogenesis of rosacea (Yamasaki et al.*, 2007) provides a possible molecular mechanism by which mite infestation of facial skin may contribute to disease symptoms. Yamasaki and colleagues (2007) determined that individuals with rosacea express higher levels of cathelicidin antimicrobial peptides in their facial skin than do unaffected controls and that these cathelicidins are processed differently in the skin of rosacea patients, creating a proinflammatory environment. Demodex mites present in sebaceous follicles might thus be expected to induce cathelicidins in the surrounding skin as part of the innate immune response to this invader and thereby contribute to rosacea's clinical manifestations of erythema and pustules.]*

CONCLUSION

[The Kligman–Christensen skin surface biopsy technique appears to provide a practical, reproducible and quantitative assessment of Demodex infestation in human facial sebaceous follicles, appropriate for assessing the putative role of these mites in various skin diseases. The clear and complete description, rescued posthumously from Kligman's unfinished work with the assistance of his coauthor and his wife, Dr. Lorraine Kligman, is a tribute to his persistence and to the confidence, friendship, and loyalty he inspired in his collaborators (Supplementary Figure 3).

Based on his comprehensive review of the literature, his own clinical experience and experimental data, and, perhaps most critically, his nearly infallible instincts, Dr. Kligman suggested that disorders caused by "demodecicosis" include (i) rosacea, especially the ocular, granulomatous, and papulopustular subtypes; (ii) papulopustular eruptions with a "sebaceous distribution" in immunocompromised patients; and (iii) perioral dermatitis, particularly cases secondary to topical corticosteroid use. As old-fashioned as mites may seem, and as low-tech is their removal from the follicle with Krazy Glue on a glass slide, the reader is cautioned that one of the great minds in dermatology suspected Demodex to be an unindicted coconspirator in several still poorly understood skin disorders. One could do worse than to further consider the possibility.]

CONFLICT OF INTEREST

Dr. Christensen states no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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